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Short Synopsis
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Title: BIOLOGICAL CONTROL OF *Alternaria brassicae* CAUSING BLIGHT DISEASE OF INDIAN MUSTARD, *Brassica juncea* (L.) THROUGH MICROBIAL CONSORTIUM

***DEPARTMENT OF* Biotechnology**

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Declaration

This is to declare that this short synopsis reflects the research work design and its contents are the original piece of work, as per guidelines, grammatically correct and free from plagiarism. This has been prepared under the guidance and supervision of my supervisor/joint-supervisor.

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Recommendation

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ABSTRACT

Oilseed brassicas commonly known as rapeseed-mustard is the second largest oilseed crop of the world. Rapeseed –mustard growing in India is greatly affected by numerous fungal diseases caused by various pathogens. One of the highly common and devastating disease *Alternaria* blight is caused by dreadful fungi, *Alternaria brassiace*. The Pseudo-fungi reduces the yield as it damages the follicles affecting the oil quality. Appearance of necrosis on leaves, stem and siliquae are the characteristics for the infection of the plant by the fungi. Humid weather greatly affects the growth of fungi, hot and dry conditions are unfavorable for its growth due to absence of moisture. In India the cultivated varieties of rapeseed-mustard shows no evidence of resistance against this acute disease. Management of *Alternaria* blight by diverse chemicals has been reported by various pathologists. Excessive use of synthetic chemicals is hazardous for the plant as well as for the environment. Studies are being carried all over the world to find the ecofriendly solution against the pathogen. Therefore, this study aims at reduction of *Alternaria* blight disease through bio control agents and to increase in quality and yield of *brassica* crop.

Key words: *Alternaria* blight, Rapeseed-mustard, Bio control agents

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1. INTRODUCTION

Oilseed crops play an important role in agriculture economy of India which constitutes the second largest agriculture product next to food grains in the country **(Mathal *et al.*, 2011).** In India, Rapeseed –mustard is a significant edible oilseed crop. This crop accounts 26.1% of the total oilseed production. According to statistical data every year 59.93 m tones of rapeseed-mustard is produced over 30.74 m hectare land worldwide, out of which 7.67 m tones are produced in India over a land of 6.5 m hectare **(GOI 2011).** Rabi crops are the crops grown in winter, flowering of these crops are expected in November, December that lasts to January and early February followed by harvesting in the month of March, April and May . Mustard being one of them and occupies the major part of oilseed production **(ICAR, 2011).**

World Production of rapeseed-mustard has increased faster over past two decades than any other oilseed. At present, rapeseed has become the second largest produced oilseed in the world after soybean. Rapeseed-Mustard is an important oilseed crop of India. Some major producing states of India contribute around 86.9% of the total production. In Haryana it is cultivated in districts like Faridabad, Bhiwani, Hissar, Mahendergarh.

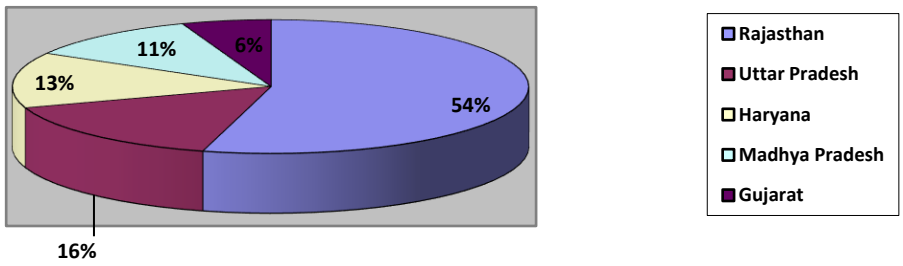

Figure.1: Rapeseed-Mustard Producing States of India
(Commodity insights yearbook, 2010)

In India seven major oilseeds belonging to the family Brassicacea are grown, these are:

- *Brassica juncea (L*) commonly called as Indian mustard or rye.
- *Brassica campestris* has three ecotypes named oleifera ,toria and tora
- *Brassica napus* commonly called as Ethopian Mustard
- *Brassica carinata* commonly called as karan rye

Oilseed brassica face major obstructions during their production. Enemies of this crop include pests, environmental factors like humidity, sowing dates and plant pathogens. Pathogens cause major destruction to the plant and hence reduce productivity. *Alternaria* blight is considered as one of the major consequences on the basis of their wide distribution and yield losses caused to Brassica spp. all over the world including India. Production of mustard in India, is drastically reduced due to presence of *Alterneria brassicae* in the fields.This disease is much prevalent in Haryana and cause considerable yield loss **(Meena *et al, 2010*)**. The disease may cause yield loss of 35-38% in mustard but in susceptible cultivators the losses may be as high as up to 70% .Yield is lost due to appearance of dark black spots on the fruit bearing branches and pods that leads to premature ripening of pods and shedding of leaves **(Meena et al., 2010).**

Figure 2: Stem lesions by *Alternaria brassiace* (https://www.canolacouncil.org)

Figure 3: Spots on leaves due to blight disease (https://www.canolacouncil.org)

It produces distinct symptoms on foliar parts of the plant **(Meena et al, 2005);** the infection starts with leaves, older leaves being close to the soil get affected first which further moves to the

upper and middle leaves which results in the defoliation of the lower leaf. The infection then widens with the visibility of black spots on the stem and siliquae. The infection further leads to complete blackening of the siliquae. Spots on leaves, stem and siliquae appear on the plant when infected by pathogen. Conidiophores in Alternaria brassica produce conidia (asexual spores) which measure around 160-200×10^{-6} meter.

Figure 4: Single conidia of *Alternaria brassiace* (http://www.padil.gov.au)

Figure 5: Multiple conidia of *Alternaria brassicae* (http://web2.mendelu.cz)

The process of sporulation takes place within the temperature range of 8 to 24 degree Celsius. The disease index is higher in late sown crops resulting in the increase in severity among the leaves **(Meena *et al*., 2002a)** and pods **(Sandhu *et al*., 1985).**

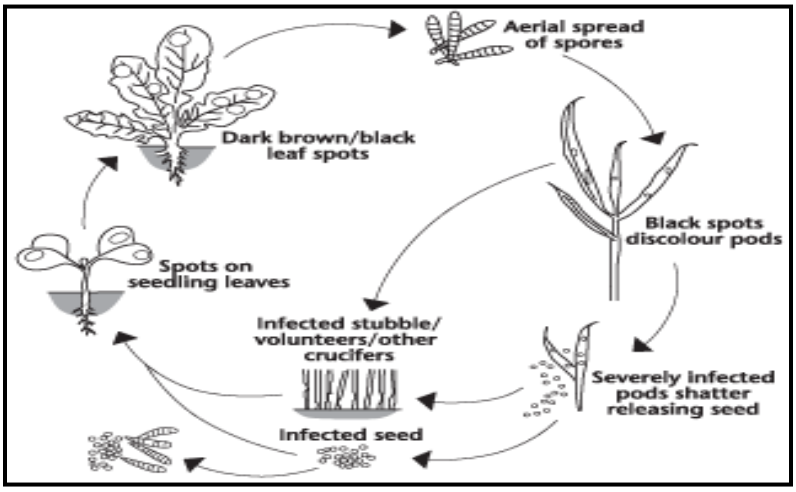
Aerial spread of spores
Dark brown/black leaf spots
Black spots discolour pods
Spots on seedling leaves
Infected stubble/ volunteers/other crucifers
Severely infected pods shatter releasing seed
Infected seed

Figure 6: Disease cycle of *Alterneria brassicae*

This acute infection caused by pathogen can be resolved by chemical management, and this is the most commonly used practice. Biological control is a promising tool to maintain agricultural

production while reducing the use of chemical pesticide. It is a cascade of events which begins with sensing of host remotely, followed by growth stimulation, finally developing relation between fungal antagonist and host surface. This provides an opportunity for intensive research finding against the pathogen biologically by using bio control agents, plant extracts and other biotechnological techniques.

2. LITERATURE REVIEW

Rapeseed-Mustard is one of the most important oil seed yielding crops in India. History states that brassicas are the earliest crops domesticated by man. The crop is affected by various biotic and abiotic factors. Among biotic stress Alternaria blight caused by *Alternaria brassicae* (Berk.) Sacc is one of the most widespread and destructive disease throughout India **(chatopadhyay., 2008).** In Haryana leaf spot of Indian mustard caused by *Alternaria brassicae* has been reported by **A.S Rathi (2010)**.

Alternaria, a member of Deuteromycetes is a facultative parasite. **Chauhan *et.al*, (2009)** reported that many saprophytes and endophytes species comprise the *Alternaria* genus. It cause severe infection on most of the family *Brassicaceae* as a result of which the quality and quantity of crop decreases **(Jackson et al., 2000).**The sternness of Alternaria blight of oilseed Brassicas is greatly affected by weather; the Brassicas gets affected by variability of temperature and relative humidity **(Awasthi and Kolte, 2003)**. These authors further found that 91.5% of relative humidity results in large number of mature spore production in 24 hours. According to their studies the infected plant debris is also hazardous because it acts as a major source for further disease development as the viability in internal mycelium of this fungus can last up to 12 years. This is one big concern for crops planted in recent harvested fields. **Sangwan and Mehta, (2007**) reported the areas with high relative humidity and temperature increases the disease index.

Alternaria brassicae produces a host-selective pathotoxin ‘dextruxin B (**Pedras and Smith, 1997)**. **Christopher (2008)** stated that necrotrophic lifecycle of Alternaria pathogen is facilitated by production of prolific toxins. **Meena and Awasthi (2010)** reported *Alternaria brassicae* produces cellulase enzymes and toxins.

Management of *Alternaria* blight is done only by balanced fertilizers till date. Mancozeb has been of great importance as it significantly reduces the disease severity **(Meena *et al*., 2004)**.

Fungicides are synthetic chemicals which are very harmful to the environment and their regular use could result in the development of resistance of fungal pathogens **(Meena et al., 2011).** To overcome this problem and to reduce the deleterious effects caused by these fungicides the focus these days have been shifted to bioremediation which includes production of new bio control agents and the use of other novel biotechnological tools **(Abha agnihotri, 2009).**Biological control of the disease is more favorable and safe than using synthetic fungicides **(Emmert and Handelsman, 1999).**The soil pool is diversely splendid with various useful microorganisms, it acts as innumerable post of bio control agents **(Stabb *et al*., 1994; Shoda, 2000)** The bio control mechanism acts on the basis of mode of action of the microbe, its root colonization, and antibiotics released by the microbe and also its antagonistic nature **(Shoda, 2000., Whipps, 2001).** Pseudomonas spp. is known to secrete a range of siderophores under iron stress condition. **Weller DM, (1998**) found that the range of special siderophores is released by *Pseudomonas spp., Trichoderma sp.,* can also be used as a biocontrol factor and have stronger antagonistic action (**Ding WL, Cheng HZ & Chen J (2003**).*Trichoderma* is a very useful fungus for industry and biocontrol and a bane to other fungi. *Trichoderma* spp. is present in nearly all soils and other diverse habitats.

However, reports on bio control of pathogen on this plant were found to be meager. The technology of commercialization is still in its initial phase **(Anonymus., 2013**). No proof of effective resistance against this deadly pathogen has been found in any host (**Meena *et al*., 2011, Kolte., 1985**).Since the disease is very important in causing economic loss of yields of mustard crop, developing and using effective management options is unquestionable. Using good cultural practices and developing biological control measures. It is very important as these are specific, efficient and environmentally safe.

3. DESCRIPTION OF THE STUDY:

Brassica juncea commonly known as Indian mustard belongs to the family brassicacea (cruciferae). This crop is of great importance as it is commonly used as vegetable and oilseed commodity but it has several other benefits as well other than these like it also acts as a source of fodder, manure. Mustard being one of the important oilseed crops in India is used extensively, $2/3^{rd}$ amount of the total oil produced by mustard seeds are used for edible purpose in the county.

The intended demand for oilseeds is around 34 million tons by the year 2020 expected to be met by rapeseed-mustard. Despite of applying the most extensive techniques to increase production, we are unable to meet the supply demand. One of the major reasons for this is the plant gets greatly affected with deadly pathogen whose infection results in the massive yield loss when uncontrolled.

Alternaria brassicae is a treacherous plant pathogen which causes a dreadful disease name *Alternaria* blight mustard crop. The pathogen is profoundly affected by weather conditions chiefly with the moisture content in the air and soil. The genomic sequence of the pathogen is still unclear. Earlier workers only reported management of oilseed Brassica disease by fungicides. Fertilizers like mancozeb and iprodione are used for disease management. Resistance could be developed against fungal pathogen with their continuous use.

Till now ecofriendly techniques like bio control agents have not been used to control the pathogen and the severity caused by it in the plant. The National Farmer Policy (2007) has strongly recommended the promotion of bio pesticides for increasing agricultural production, sustaining the health of farmers and environment. The present study aim to report identification of effective bio agents and their consortium for the management of the disease of mustard in Haryana.

4. OBJECTIVE OF THE STUDY

This work is focused on the development of consortium of bio control agents to eradicate *Alternaria* blight disease of *Brassica juncea* (Indian mustard) with the following objectives:

1. To conduct survey, identification and isolation of *Alternaria brassiace* in the fields of mustard in and around Faridabad in Haryana.

2. To study the effect of various environmental factors on the growth of *Alternaria brassicae*.

3. To conduct the *in-vitro* antagonistic studies of isolated bio control agents against *Alternaria brassicae*.

4. To assess the ability of bio control agents to produce secondary metabolites.

5. To characterize the selected bio control agents at molecular level.

6. To test the efficacy of the selected bio control agents on the growth of *Brassica juncea* under greenhouse conditions.

5. METHODOLOGY TO BE ADOPTED

5.1 Survey, identification and isolation of *Alternaria brassicae*:

The fungal pathogen (*Alternaria brassicae*) will be isolated from diseased leaves of *Brassicae juncea* by using blotter technique (**Chauhan *et al*., 2009, de Tempe, 1953**). The infected leaves of *Brassicae juncea* will be procured by survey of farmer fields from Faridabad region. The pure culture thus obtained will be transferred on PDA and maintained at 4°C in a refrigerator. Pathogenicity tests of pathogen isolates will be done in green house conditions.

5.2 Studies on the effect of various environmental factors on the growth of *Alternaria brassicae*:

The various nutritional and physiological variability of *A. brassiace* will be carried by as described by **Meena *et al*., (2011).**

5.3 Evaluation of various Biological control agents (BCAs) against Alternaria *brassicae*:

Isolation and purification of *Pseudomonas and Trichoderma* strains will be done by using blotter technique (**Chauhan *et al*., 2009, de Tempe, 1953).** The culture will be transferred to their respective slants. The slants will be stored and maintained at 4°C in a refrigerator.Biochemical characterization of the bacterial strains will be carried out according to Bergey's Manual of Determinative Bacteriology **(Holt *et al*., 1994).** *Trichoderma* will be identified on the basis of their morphological characters (**Abdel-Fattah GM et al, 2007).** The biocontrol agents will be tested as an antagonist against *Alternaria brassicae* on potato dextrose agar plates using the dual culture technique (**Skidmore and Dickinson. 10976**).

5.4 Assesment of biocontrol agents to produce secondary metabolites :

Detection of siderophores: Production of siderophores will be estimated qualitatively on chrome-Azurol S agar medium (**Srivastava *et al*., 2013)**. HCN Production: HCN production will be tested by method of Castric and Castric, (**Lanteigne *et al*., 2012).** IAA Production: Indole acetic acid production will be tested according to **Gordan and Weber (1951)** and **Bric *et al.* (1991).**Phosphate Solubilization: Phosphate Solubilization will be performed by doing spot inoculation of test organism on Pikovskaya's medium.

5.5 Molecular characterization of the Biocontrol agents:

The selected isolates will be sub-cultured in continuous light and dark conditions in incubator for 10 days. The DNA of all the collected isolates will be extracted. Total bacterial and fungal DNA will be extracted by CTAB methods as described by **Murray and Thompson (1980).** Sequencing and bio informative analysis will be carried out later on.

5.6 Evaluation of the selected bio control agents on the growth of *Brassica juncea* under greenhouse conditions:

Pot trials will be conducted to evaluate the efficacy of developed consortium under on-site (*in situ*) environmental conditions. Various physico-chemical parameters of soil will be determined. The various parameters for Mass scale culture of Bioinoculant formulation will be standardized. Application of Bioinoculant through carriers in field will be done.

6. PROPOSED OUTCOME OF THE RESEARCH

The expected outcome of this work is to control the disease caused by the fungal pathogen by using environment friendly techniques. Popularization of bio pesticides is very slow as compared to chemicals and only 2% bio pesticides are available. Many good microbes are much exploited and their success rate is also remarkably high, hence useful microbes will be used to cure the Alternaria blight disease of Indian mustard caused by *Alternaria brassicae.* These microbes will be delivered to the plant cell by various processes. According to recent surveys done, market of bio pesticides is expected to get double by 2020. Hence, there is considerable room for bio pesticide to gain attention in the market. Therefore the aim of this study is to search for potential

microbes which could show antagonistic activity against the causal pathogen and to ensure the survival of these microbes in experimental field conditions.

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